

Isolation, purification and characterization of biofilm forming *Staphylococcus aureus* from waste water in Dhaka city, Bangladesh

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Abstract: The study was carried out for isolation, purification and characterization of biofilm-forming bacteria *Staphylococcus aureus* from waste water. The waste water sample was collected for the potential biofilm producing bacteria from the site of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka on March 1, 2022. The isolate was identified by using colonial characteristics on different selective media and biochemical tests. The microscopic observation of the isolate was gram-positive, purple in colour, grape like clusters and single or two-pair colonies on the screen. To identify the bacteria, eight biochemical tests were performed. Among them, six tests were showed positive results. Morphological and biochemical characteristics confirmed that the bacterial isolate is *S. aureus*. Biofilm formation assay was carried out by tube adherence method (TM) and Congo red agar method (CRA). Antibiofilm profile of identified bacterial isolate was checked through culture and sensitivity test by Kirby-Bauer method with Mueller-Hinton agar plates. Sensitivity of all bacteria were checked against 14 commercially available antibiotic discs. The isolate was found to be resistant against Imipenem (10) and Kanamycin (30). The antibiotics used in hospitals for patient care may cause antibiotic-resistant bacteria to grow in hospital waste water and spread to environments outside of hospitals via drainage system. Based on the findings of this study, it was affirmed that the biofilm producing *S. aureus* bacteria can survive at highly contaminated area and show resistant to different antibiotics.

Keywords: Biofilm, *Staphylococcus aureus*, TM, BPA, CRA, Imipenem, Kanamycin

Introduction

The relatively ill-defined microbial community that is attached to a surface or any other hard, non-shedding material and is dispersed randomly in a shaped matrix or glycocalyx is referred to as a biofilm. Microbes and other organic and inorganic materials

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are bound together in a polysaccharide matrix in the lower layers of a biofilm. A loose, amorphous layer that penetrates the surrounding medium is located above it. There are stationary and dynamic sub layers in the fluid layer that surrounds the biofilm. EPSs (extracellular polymeric substances) give biofilm their three-dimensional form and stabilize it. Channels that function as a circulatory system are frequently permeated by biofilm, enabling the bacteria to exchange nutrients, enzymes and signals, get rid of potentially harmful metabolites and exhibit improved metabolic cooperation. Bacteria can colonize new surfaces or substrates thanks to the dispersal of biofilm, which completes the development cycle (Bogino *et al.*, 2013)¹.

The complex process of biofilm formation involves several distinct phases, starting with the absorption of a conditioning film made of bacterial and host molecules onto the tooth surface right after tooth eruption or tooth cleaning. Following this absorption, bacteria are passively transported by weak long-range forces of attraction. Strong, short-range forces produced by covalent and hydrogen bonds lead to irreversible attachment. By auto aggregation (attraction between members of the same species) and coaggregation (attraction between members of different species), the primary colonizers create a biofilm. coaggregation causes the formation of various morphologic structures, including corncobs and rosettes, as well as the functional organization of plaque bacteria. Currently, the microenvironment is facultative, anaerobic rather than aerobic or capnophilic (SCIENCEDIRECT.com)². A mature mixed-population biofilm is created when the attached bacteria multiply and secrete an extracellular matrix. Biofilms offer the species that are colonizing against rival microorganisms, environmental elements like host defense mechanisms and potentially toxic substances like lethal chemicals or antibiotics.

Antibiotic resistance among organisms in biofilm is 1000-1500 times higher than that of planktonic organisms. The mechanisms underlying this heightened resistance vary depending on the species, antibiotic and type of biofilm being grown. Bacteria's nutritional status, growth rate, temperature, pH and previous exposure to low concentrations of antimicrobial agents are thought to have an impact on their antibiotic resistance. The slower rate of bacterial species growth in a biofilm, which reduces their susceptibility to bactericidal antibiotics, appears to be another significant mechanism. Antibiotic diffusion can be resisted by biofilm matrix. Strongly charged or chemically highly reactive agents, for instance, may be unable to penetrate the biofilm deeper zones because the biofilm serves as an ion exchange for the removal of such molecules. Within a biofilm, "super-resistant" bacteria have been found that have multidrug

-resistant pumps that can extrude antimicrobial substances from the cell. The process provides defense against antibiotics that target cell wall synthesis because these pumps move the antibiotics outside the outer membrane. Antimicrobial must be used in the treatment of infections linked to biofilm, and the observations mentioned above are crucial.

Staphylococcus is a gram-positive spherical bacterium that frequently causes surgical and skin infections, respiratory illnesses, and food poisoning. Staphylococci were first identified in pus cells from a surgical abscess in a knee joint in 1880 by Scottish surgeon Sir Alexander Ogston (Licitra, 2013)³. The bacterium is roughly 0.8 mm in diameter, or 60 times smaller than the width of a human hair. An opportunistic pathogen is *S. aureus*. Under typical circumstances, it is not dangerous. However, if given the chance, such as when the skin or mucous membrane is damaged, it can seize the moment and spread an infection, which can occasionally have grave consequences. Food poisoning can also be caused by *S. aureus*. Some *Staphylococcus aureus* strains have the ability to produce staphyloxanthin, a carotenoid pigment with a golden hue. The primary way in which this pigment functions as a virulence factor by acting as a bacterial antioxidant and assisting the microbe in dodging the reactive oxygen species that the host immune system uses to kill pathogens. Staphyloxanthin-deficient mutant strains of *S. aureus* are less likely than pigmented strains to survive incubation with an oxidizing agent like hydrogen peroxide. When exposed to human neutrophils, mutant colonies are quickly destroyed while many of the pigmented colonies survive. *Staphylococcus aureus* can flourish in conditions with a pH range of 4.2 to 9.3 and up to 15% salt content. Temperatures that would kill the bacilli can't kill enterotoxins. Sensitive to dry heat treatment at 160-170°C for at least an hour, but not to moist heat treatment. Staph infections are commonly treated with antibiotics such as cloxacillin and cephalixin. MRSA is treated with vancomycin, which is given intravenously. 70% ethanol, chlorhexidine, 1% sodium hypochlorite, 2% glutaraldehyde, 0.25 benzalkonium chloride and formaldehyde all toxicants that can harm an organism. Due to its antibiotic resistance and the rising prevalence of indwelling medical devices, *Staphylococcus aureus* has emerged as a clinically significant pathogen. The crude mortality rate of *S. aureus* infections in the US is 25% and hospitalizations due to these infections typically result in hospital stays, deaths and medical costs that are about twice as high. Once established *S. aureus* biofilms are resistant to antimicrobial therapy and the host response and as a result, they are the etiological agent of numerous recurrent infections (Kloos and Bannerman, 1994)⁴.

Systemic combined with topical antibiotic treatment can provide better results against biofilm infections for patients that are amenable to topical treatment with high concentrations of antibiotics, such as antibiotic inhalation or direct administration for airway biofilm and bladder irrigation with high concentration of antibiotics against biofilm urinary tract infections. (Foxman, 2010)⁵.

It was found that production of bacteriolytic enzymes is a general property of staphylococci^{6, 7}. It was possible to subdivide over 1,000 human *Staphylococcus* isolates into six groups (lyogroups) based on their bacteriolytic activity patterns analyzed through a simple *in vivo* assay⁸.

The study about the further research on the environment are very important. Therefore, the objectives of this study are as follows:

1. Screening of biofilm forming *Staphylococcus aureus* isolated from waste water specimens.
2. Isolation of *Staphylococcus aureus* biofilm producing bacteria using Mannitol Salt Agar and Baird Parker Agar.
3. Characterization of the isolate through:
 - i) Morphological analysis, microscopic observation and biochemical tests.
 - ii) Antibiotic susceptibility test.
4. Formation of biofilm from the isolate using the Tube Adherence Method (TM) and Congo Red Agar (CRA) method.

Materials and Methods

The experiments were conducted in 2022 at the Industrial Microbiology Research section of the Bangladesh Council of the Scientific and Industrial Research (BCSIR), Dr. Quadrat-E-Khuda Road, Dhaka.

Collection and processing of samples

Following guidelines of Clinical and Laboratory Standard Institute (CLSI), under aseptic conditions, sample was collected from the waste water of BSMMU, Dhaka with a sterile spatula. The specimen was then placed in a sterile nutrient broth in a screw capped test tube. It was then promptly brought to the Industrial Microbiology Research section, IFST, BCSIR within 1 hour for additional processing and examination.

Isolates

Staphylococcus aureus was collected from waste water.

10g of each sample was weighed and diluted with 90 ml of pre-enrichment buffered peptone water homogenized and incubated at 37 °C for 24 hours.

↓

1 ml from this pre-enrichment culture was transferred to 9 ml enrichment broth, Tryptic Soy broth and incubated at 37 °C for 24 hours.

↓

100 µl of the enrichment culture was plated onto Baird parker agar. Plates were incubated at 37 °C for 24 hours.

↓

Morphologically in BPA, *Staphylococcus spp.* grows as round, raised, sticky and shiny. Dark grey colonies having complete edges. Typical colonies were taken as possible *Staphylococcus spp.*

Sterilization

All tools and glasswares were sterilized by autoclaving at a pressure of 15 P.S.I for 20 minutes.

↓

The autoclave was used to sterilize all growing media and solutions for 15 minutes at 15 P.S.I.

↓

Before inoculating the plates with microorganisms, fresh agar plates were made at room temperature, kept at 4°C until use.

↓

Inoculating plates then dried at room temperature under aseptic conditions.

Growth of microorganisms

Every culture was inoculated in an aseptic environment.

↓

The organisms were inoculated in a laminar airflow.

↓

Using static growth conditions.

↓

The inoculated solid and liquid media were incubated at 37°C.

Preparation of solution

An electrical balance was used to accurately weigh the components of various solutions. A digital pH meter was used to determine the pH of various buffer solutions, media and so on.

Cultures, reagents and solutions maintenance

The preservation and maintenance of various materials, such as microbial strains, heat labile chemicals or reagents were kept in a refrigerator at 4°C.

↓

The pure bacterial strains were routinely maintained on Nutrient Agar.

↓

The pure bacterial cultures were kept at 4°C until they were used.

↓

Every two months, fresh agar was used to prepare subcultures.

↓

The bacterial culture was tested on a Nutrient Agar plate before use.

↓

This plate was then designated as a stock plate, from which further investigation was conducted.

Growth Media

Media used for growth and confirmation of strains

Nutrient Agar (NA)

Nutrient Agar media was commonly used for routine bacterial growth and subculture of *Staphylococcus aureus* usually in petridishes to ensure the purity before performing any biochemical or serological tests.

Nutrient Broth (NB)

Nutrient broth media was used to grow *Staphylococcus aureus* in culture bottles or tubes before antibiotic sensitivity test. It was also used to keep *Staphylococcus aureus* stocks alive.

Luria Bertani Agar (LBA)

Luria Bertani agar media was commonly used for routine bacterial growth and cultivate of *Staphylococcus aureus* in petridishes prior to use.

Luria Bertani (LB) Broth

It was widely used to rapid and good growth of *Staphylococcus aureus* in culture bottles or tubes. It was also used to keep *Staphylococcus aureus* stocks alive. *Staphylococcus aureus* was isolated using BPA and MSA media.

Baird-Parker Agar (BPA)

In BPA, Egg Yolk Telluride Emulsion was added as a supplement to identify Coagulase positive Staphylococci. It was distinguished by the formation of black, shiny, convex colonies surrounded by an egg yolk lighting halo.

Mannitol Salt Agar (MSA)

Mannitol Salt agar was used to isolate and identify *Staphylococcus aureus* from clinical and non-clinical samples. This medium was applied to confirm the presence of *Staphylococcus aureus*, since Coagulase positive Staphylococci was produced yellow colonies and a yellow medium around it, whereas Coagulase negative Staphylococci was produced red colonies and no color change of the Phenol red indicator.

Media used for biochemical identification

Dehydrated media such as Simmons's Citrate Agar (SCA), Kligler's Iron agar (KIA), Triple sugar Iron (TSI) agar, Sulphide Indole Motility (SIM) media were used for the identification of the isolate.

Identification of selected bacterial isolate

The identification of biofilm producing bacterial isolate was carried out based on morphological characteristics, gram staining and biochemical characteristics.

Observation of colony characteristics

The colony characteristics of the isolate was observed and tabulated.

Gram staining

Gram staining was performed for differentiating between two principal groups of bacteria: Gram positive and Gram negative. Gram reaction was studied by Hucker's modified method (1884)⁹.

Biochemical tests

Biochemical tests were performed to identify the bacteria according to the American Society of Microbiology's manual of methods for general Bacteriology (ASM, 1981)¹⁰. The catalase, oxidase, indole, methyl red (MR), Voges-Proscauer (VP), citrate utilization, motility, H₂S production tests were done.

Screening of biofilm forming bacteria

The screening of biofilm forming *Staphylococcus aureus* was detected by tube adherence method (TM), Congo red agar (CRA) method and Antibiotic sensitivity test (Christensen et al., 1995¹¹; Freeman et al., 1989¹²).

Antibiotic Sensitivity Test

Antibiotic sensitive and resistant *Staphylococcus aureus* colonies were identified using 14 different antibiotic discs. These were Doxycycline, Oxytetracycline, Amikacin, Netilmicin, Sulfafurazole, Enrofloxacin, Imipenem, Kanamycin, Trimethoprim, Florfenicol, Vancomycin, Cephadrine, Nitrofurantoin and Ticarcillin.

Steps performed in antibiotic sensitivity test

The process steps are listed below:

1. Sterile cotton swabs were used, each test bacterial strain was lawn cultured on properly labeled Mueller Hinton agar plates. The bacteria was grown in Nutrient broth and incubated at 37°C for 24 hours.
2. Following lawn culture, antibiotic disks were very carefully picked up from the stacks with sterile forceps and placed on the lawn culture.
3. Care was taken to ensure that the disks were well-spaced in order to prevent inhibition zones from overlapping.
4. The plates were then incubated for 24 hours at 37°C.
5. Antibiotic sensitivity was determined after incubation by measuring the diameter of the inhibition zone in millimeters.
6. Finally, the results were interpreted.

Results and discussion

Screening, isolation and identification of biofilm forming microorganisms from waste water

The results of initial screening and isolation of the bacteria *Staphylococcus aureus* from waste water sample was showed opaque zone, grey to black or brown-grey colonies on Baird-Parker agar plates (Figure 1) and golden-yellow colonies on Mannitol Salt agar plates (Figure 2) by using pour plate method. A positive result was obtained from *Staphylococcus aureus* found in the waste water sample. Cultural, Morphological and Biochemical traits were used to identify *S. aureus*.

Colony characteristics

After 24 hours, the color, form, elevation, margin, surface and optical characteristics of *S. aureus* was observed on Mannitol Salt agar and Baird Parker agar plates. Table 1 illustrated morphological characteristics for *S. aureus*. The Isolate was distinguished with Cocci, Golden-yellow, Circular and entire margin.



Figure1. Isolate of *S. aureus* on Baird-Parker agar plates.

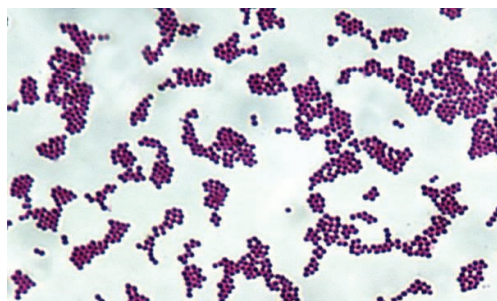
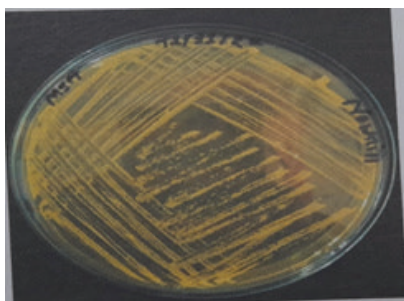


Figure 2. Isolate of *S. aureus* on Mannitol Figure 3. Microscopic observation of isolated *S. aureus* Salt agar plate.

Table 1. Morphological characteristics of the biofilm forming bacterial isolate from waste water sample.

Isolate	Size	Gram stain	Color	Form	Margin	Elevation	Surface
I	Cocci	+	Goldenyellow	Circular	Entire	Convex	Concentric

Microscopic observation

Isolated colony was introduced to gram staining and observed under microscope shown in Figure 3.

Biochemical characteristics of the bacterial isolate

Microorganism was isolated and sub-cultured for biochemical tests after spreading and streaking on the agar plates. The tests used an isolate had undergone a 24 hours fresh culture. Following subculture, a few particular biochemical tests were carried out and recorded. The possible organism was then interpreted according to the American Society of Microbiology’s manual of methods for general Bacteriology (ASM, 1981)⁹. Table 2 was showed biochemical test results for Staphylococcus aureus.

Table 2. Biochemical test results of the isolate from waste water sample.

Isolate	Catalase test	Oxidase test	Indole test	Methyl red test	VP test	Citrate test	Motility test	H ₂ S test
1	+	-	-	+	-	-	-	-

Catalase Test

The isolate was shown Catalase positive (Figure 4). The *Staphylococcus* spp. is Catalase positive.

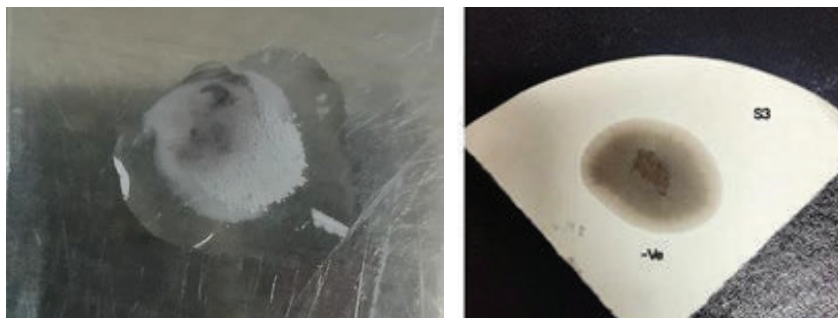


Figure 4. Catalase test of the isolate. Figure 5. Oxidase test of the isolate.

Oxidase Test

The Oxidase test is a biochemical reaction that assays for the presence of cytochrome Oxidase, an enzyme sometimes called indophenols Oxidase. In the presence of an organism that contains the cytochrome Oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product. The isolate was shown in (Figure 5) was Oxidase negative.

Indole Test

Indole test was used to determine the ability of an organism to split amino acid tryptophan to form the compound Indole. After addition of kovac's reagent, a positive result had red layer at the top due to the production of Indole and negative result has a yellow/brown layer. The Presumptive isolate was shown in (Figure 6) was Indole negative.

Methyl Red Test

Five drops of the pH indicator methyl red were added to the tubes. The tubes were gently rolled between the palms of the hands to disperse the methyl red. The isolate was showed (Figure 7) red color represented the positive test.

Voges-Proscauer Test

The Voges-Proscauer test was used to determine if an organism produces acetylmethylcarbinol from glucose fermentation. If present, acetylmethylcarbinol is converted to diacetyl in the presence of α -naphthol, strong alkali (40% KOH), and atmospheric oxygen. A positive reaction was indicated by pink-red color at the surface and negative reaction was indicated lack of a pink-red color. The isolate was showed (Figure 8) negative result in VP test.

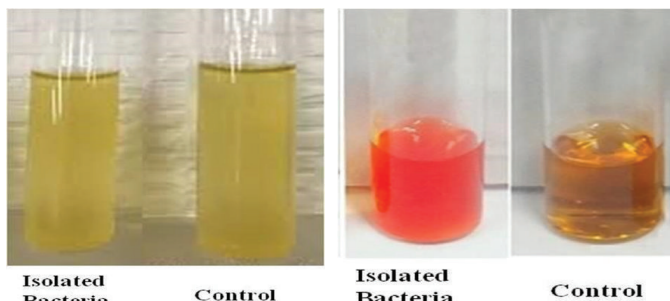


Figure 6. Indole test of the isolate. Figure 7. Methyl Red test of the isolate.

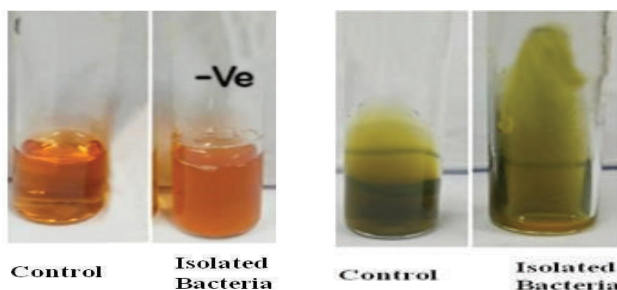


Figure 8. VP test of the isolate. Figure 9. Citrate Utilization test of the isolate.

Citrate Utilization Test

A positive result for this test was indicated by color change in the medium from green to blue. When microorganisms utilized citrate, they removed the acid from the medium, which raised the PH and turned the PH indicator (bromothymol blue) from green to blue. Negative results were indicated by no growth and no color change. The isolate was indicated (Figure 9) citrate negative.

Motility Test

Motile bacteria can move through semisolid, soft motility agar and their growth clouds the medium. Non motile organisms will remain still and only grow near the site of inoculation. Motility test is used to determine the motility of bacteria. The isolate was showed negative result for motility test (Figure 10).

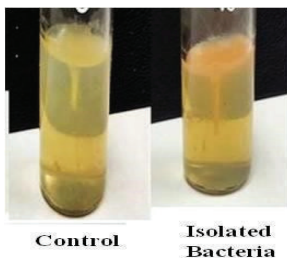


Figure 10. Motility test of the isolate.

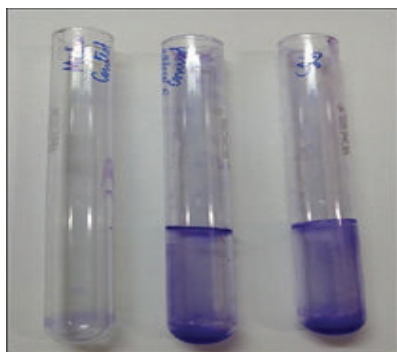


Figure 11. Screening of biofilm formation by TM.



Figure 12. Screening of biofilm by CRA method.

The Tube Adherence method (TM)

This qualitative method is used to detect the organism. In this method, the isolate was showed visible lining of the wall and bottom of the tube by a film was considered as positive. The results were scored visually as moderate or strong biofilm producers (Figure 11).

Cong Red Agar method

The simple qualitative method is used to detect phenotypic identification of biofilm producing strains. The Brain Heart Infusion (BHI) agar medium was showed black colonies with dry crystalline morphology and it was considered positive for biofilm producing organism (Figure 12).

H₂S Test

This test is used to determine the organism converts sulfides from sulfur-containing compounds to hydrogen sulfide gas. The presence of hydrogen sulfide was indicated by the black color.

Antibiotic Sensitivity Test

An antibiotic sensitivity test was carried out after the identification and confirmation of the organism. For the waste water sample isolate, fourteen antibiotics were used. These were Doxycycline, Oxytetracycline, Amikacin, Netilmicin, Sulfafurazole, Enrofloxacin, Imipenem, Kanamycin, Trimethoprim, Florfenicol, Vancomycin, Cephadrine, Nitrofurantoin and Ticarcillin. By measuring the zone of inhibition in millimeters for each antibiotic, it was possible to identify the isolate's sensitivity and resistance patterns (Figure 13). Among all the antibiotics, the isolates of *Staphylococcus aureus* were resistant to Imipenem and Kanamycin.

Table 3 shows the interpretation of each bacterium's resistance or susceptibility to antibiotics.

The results of initial screening and isolation of the bacteria *Staphylococcus aureus* from waste water sample was showed opaque zone, grey to black or brown-grey colonies on Baird-Parker agar plates and golden-yellow colonies on Mannitol Salt agar plates by using pour plate method (Table 1). The isolates of *Staphylococcus aureus* were shown catalase positive and cytochrome oxidase activities (Figure 4 and 5). These findings were supported by Satta *et al.*, (6,7).

To detect the *Staphylococcus aureus* two methods broadly used for the phenotypic identification. (de Castro Melo *et al.*, 2013)¹³ The Tube method (TM) is used as a screening test. (Mishra *et al.*, 2015) Although TM is one of the simplest techniques employed, the presence of visible errors should be considered with the method. (Mathur *et al.*, 2006)¹⁴.

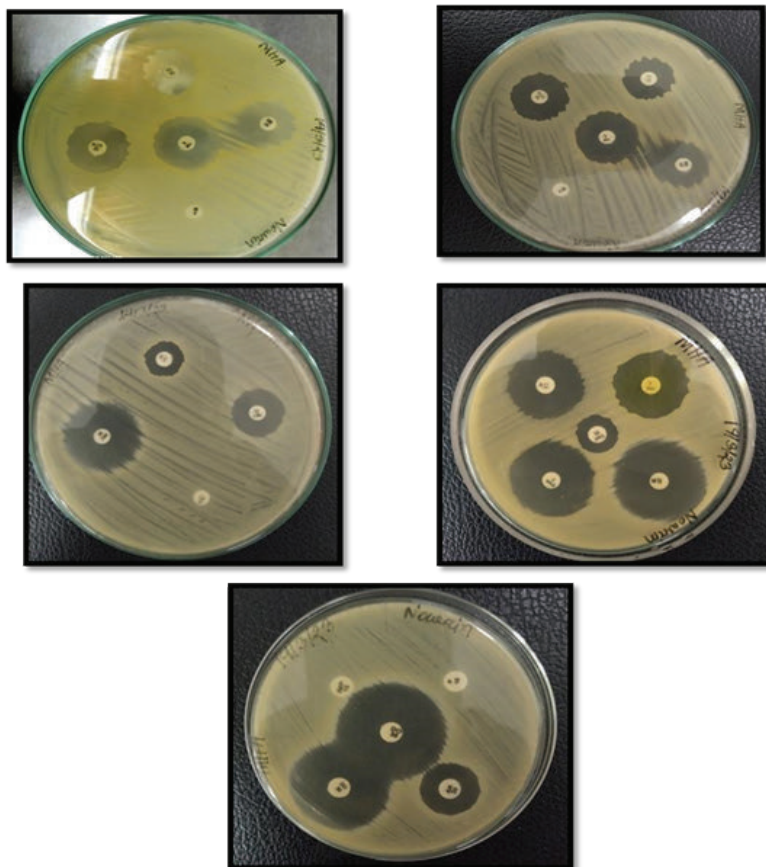


Figure 13. Antibiotic susceptibility pattern of biofilm producing bacteria.

Moreover, TM is cost effective and easily available which has to be taken under consideration in a developing country like Nepal. Therefore, TM may be used for the screening of biofilm during routine laboratory work at the general hospital. (Neopane et al., 2018)¹⁵. The Congo red agar (CRA) method allows for the direct analysis of the colonies and the identification of slime forming. This is not a quantitative assay because it is based on a subjective chromatic evaluation. The strains that score positive during the test have black spikes on red colonies which remain unchanged in color (de Castro Melo et al., 2013)¹³. Therefore, the adherence property of biofilm producers was graded as strong, moderate and weak in both methods used in this study (TM and CRA).

Table 3. Antibiotic susceptibility pattern of biofilm forming bacteria isolated from waste water sample.

Code No.	Name of Antibiotics	Disc Concentration (µg/disc)	Interpretation of results
			mm
DO	Doxycycline	30	30
OT	Oxytetracycline	30	30
AK	Amikacin	30	17
NET	Netilmicin	30	20
SF	Sulfafurazole	300	22
ENR	Enrofloxacin	5	30
IMI	Imipenem	10	R
K	Kanamycin	30	R
TM	Trimethoprim	5	23
FFC	Florfenicol	30	30
VA	Vancomycin	30	15
CE	Cephadrine	30	25
F	Nitrofurantoin	300	24
TC	Ticarcillin	75	20

R = Resistant

In this present study, the isolate was showing strong adherence. In respect of antibiotic susceptibility test, the isolate was resistant to imipenem and kanamycin. Besides, the isolate was showed different zone diameter to other antibiotics such as Doxycycline, Amikacin, Oxytetracycline, Florfenicol, Vancomycin, Netilmicin, Nitrofurantoin, Trimethoprim, Enrofloxacin, Sulfafurazole, Cephadrine and Ticarcillin on Mueller Hinton agar plates.

Numerous studies have discovered that *Staphylococcus aureus* is largely present in waste water. According to the CLSI criteria, the substance known as vancomycin-intermediate (VISA) was created as a result of *S. aureus* adapting to the antibiotic vancomycin, which had long been the last line of defense against MRSA infections. Vancomycinintermediate *Staphylococcus aureus* (VISA) is a disease caused by adaptive mutations added to the genes coding for control of bacterial cell physiology. Surprisingly, 64% of Vancomycin-intermediate *Staphylococcus aureus* (VISA) clinical strains had multiple mutations in the *rpoB* gene, which codes for the subunit of the RNAP core enzyme.

Staphylococcus aureus chronic biofilm-associated infections frequently result in significant increases in morbidity and mortality, especially when connected to indwelling medical equipment. This has led to a significant amount of research into *S. aureus* biofilm formation and the molecular mechanisms that underline these multi cellular structures resistance to antibiotic therapy.(Moormeier and Bayles *et al.*, 2017)¹⁶.

Conclusion

In this present study, wastewater showed presence of biofilm forming bacteria. The bacteria was screened and identified as *Staphylococcus aureus*. Globally, 17.9% to 100% of HAIs has MDR that is associated with biofilm *S. aureus*; *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* were the most common bacterial isolates responsible for HAIs in recently published studies. (Assefa and Amare., 2022)¹⁷. TM and CRA method are all effective in detecting biofilm formation. The isolate showing positive reactions can be confirmed by the quantification of slime either by Christensen's method (1995)¹¹. The study was also observed slime producing bacteria was sensitive to some of the antibiotics tested. After that biofilm was formed by the isolated bacteria and then it was partially characterized.

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E-Waste and PV Waste Management in Bangladesh (2000–2024): A Comparative Analysis of Trends, Challenges and Policy Deficiencies.

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Abstract: The rapid growth of technology and the increasing use of electronic devices have led to a global rise in electronic waste (E-waste). This trend poses serious environmental and health challenges. In Bangladesh, a developing country with a growing population and an emerging technology sector, managing E-waste has become a pressing issue. From 2000 to 2024, rapid urban growth, economic development, and digitalization have significantly increased E-waste production. This surge worsens environmental problems and threatens public health. At the same time, the use of renewable energy sources, especially photovoltaic (PV) technology, has increased to meet the country's energy needs and sustainability goals. However, the rise of PV systems presents new waste management challenges, as handling solar panel waste at the end of its life is still poorly managed. This review aims to assess the current situation of E-waste and PV waste management in Bangladesh. It will examine the regulatory frameworks, technological developments, and public awareness campaigns that have appeared in recent years. The goal is to identify shortcomings in current management practices and suggest effective strategies that consider Bangladesh's unique socio-economic context, ultimately helping to create a more sustainable waste management approach amid rapid technological changes.

Keywords: E-waste, PV waste, waste management, environment, sustainable development.

1. Introduction

Bangladesh, similar to numerous developing countries, has embraced the era of digital technology and renewable energy. From 2000 to 2024, millions of individuals have begun using mobile phones, computers, televisions, and solar power systems especially in rural regions through initiatives supported by the government and various donors. However, alongside these accomplishments exists a growing yet overlooked threat waste. While electronic waste is prominent and often addressed in public discussions, the waste generated from outdated or damaged solar panels is seldom acknowledged.

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This paper examines the two types of waste in relation to Bangladesh to guide future policy and environmental planning.

2. Definitions and Waste Components:

Waste consists of various types of discarded materials, differing in their composition and origin. The term waste is a general category that includes any unwanted or disposed items. E-waste pertains specifically to electronic waste, which are discarded electronic devices. PV waste, or photovoltaic waste, is a specific category within e-waste that refers to discarded solar panels and their parts.

Waste Type	Main Sources in Bangladesh	Hazardous Components
E-Waste	Mobile phones, PCs, TVs, fridges	Lead, mercury, flame retardants
PV Waste	Solar panels, micro-grid solar modules	Lead, cadmium, silicon fragments

3. Waste Generation Trends (2000–2024) in Bangladesh:

The increasing generation of both E-waste and PV waste necessitates a global effort to develop and implement sustainable management practices. Both E-waste and PV waste pose serious environmental and health risks if not managed properly, highlighting the urgent need for effective recycling and disposal strategies.

As the following graph (fig-1) research shows that E-waste has steadily increased from ~20 Mt in 2000 to over 45 Mt in 2024, driven by growing consumer electronics. PV waste started rising notably after 2012, following mass deployment of solar panels. PV waste is still smaller in volume but growing faster, especially as early solar panels reach end-of-life (10–15 years lifespan).

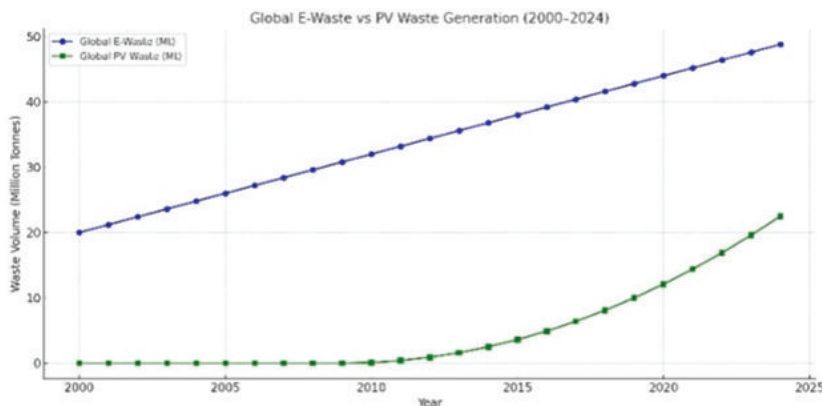


Fig. 1: Estimated amount of global E-wastes and PV waste up to year 2025

According to the report on the evaluation of E-waste generation, its environmental impacts, and the potential for resource recovery in Bangladesh, the forecast for future E-waste production has been estimated using the USEPA method (USEPA, 2009), based on end-of-life (EOL) quantities and certain assumptions regarding their percentages. Utilizing this framework, the total E-waste generated from eight selected categories has been calculated and is illustrated in Figure - 2.

From figure - 2, it is observed that estimated amount of E-waste from year 2010 is increasing significantly. At present (2025), amount of E-waste is 1.21 million ton and by year 2035 the amount will be 4.62 million ton with an annual growth rate of around 20%.

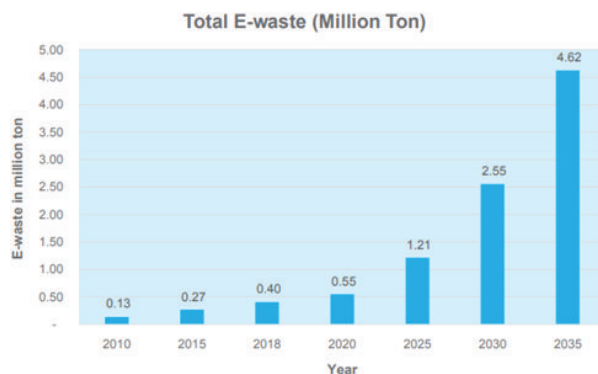


Figure-2 Estimated amount of E-wastes for Bangladesh up to year 2035

Although exact numbers for the total amount of PV waste generated in Bangladesh between 2000 and 2024 are not easily accessible as a single figure, the overall trend shows an increase in solar panel waste resulting from the rising use of solar energy in the nation. Another report shows the result in following table which also satisfy the emerging tendency of PV waste.

Year	Installed PV Capacity (approx.)	Estimated Cumulative PV Waste (tons)
2000–2003	Minimal	~0
2004–2015	Solar home systems were deployed rapidly (~4 million units by 2015)	~33,200 (total end-of-life projections by 2022)
2016–2021	Incremental increases; total around~416 MW by early 2022	~33,200 tons
2022–2024	Continued growth is anticipated, aiming for an estimated 2,500 MW by 2025 (~10% of generation)	There may be a slight increase in waste, but most end-of-life waste has not yet emerged.

The estimated figures appear realistic, given that Bangladesh is undergoing rapid development in various industries, especially in the fast-evolving IT and mobile fields.

4. Environmental Impacts:

The improper disposal of electronic waste in urban slums and informal recycling areas leads to contamination of air and water due to dangerous burning practices and the use of acids. If solar panel waste is discarded in landfills, it can seep lead and cadmium into the soil and groundwater; however, there is currently no system for monitoring or safe disposal.

Impact	E-Waste	PV Waste
Soil	Lead contamination from informal dumps	Risk of cadmium leaching
Water	Heavy metal seepage	Glass and silicon fragments in rural landfills
Health	Respiratory, neurological	Not yet studied, but potentially serious

5. Waste Management Practices:

The management of E-waste and photovoltaic (PV) waste in Bangladesh encounters major obstacles because of insufficient policies, infrastructure, and public awareness. Although the country has made progress in establishing E-waste regulations, the enforcement and implementation of these rules are still lacking. The swift rise in the number of electronic devices and solar panel installations has resulted in an increasing amount of waste, which presents environmental and health hazards.

Category	E-Waste	PV Waste
Collection	Informal collectors dominate	No dedicated collection
Recycling	Mostly informal, unsafe	None known in Bangladesh
Government Role	Draft e-waste rules exist	No PV waste mention
Take-back systems	Non-existent	Not even considered

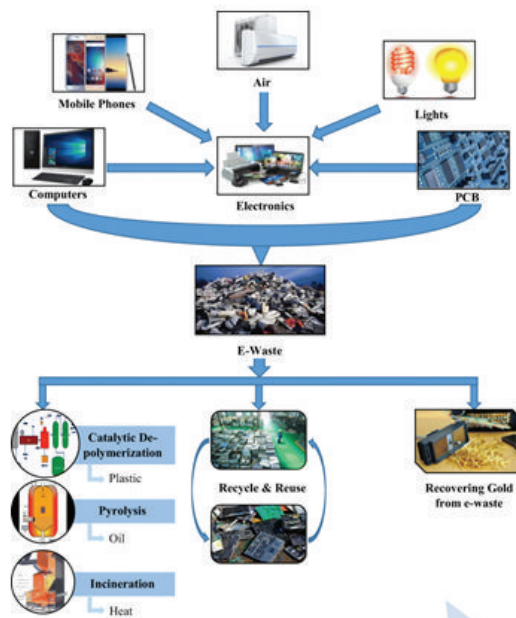
Recycling Technologies:

Disassembling electronic waste into its individual components for recycling into new items is an important process, particularly for materials such as precious metals, copper, and plastics. Meanwhile, advancing efficient recycling technologies for solar panels is vital for reclaiming valuable resources like silicon, silver, and other rare earth elements, reducing environmental harm and fostering a circular economy.

Feature	E-Waste	PV Waste
Current Practices	Informal dismantling common	Mostly landfilled
Formal Recycling	Exists in developed regions	Limited to EU, pilot programs
Material Recovery	Copper, gold, aluminum	Silver, glass, aluminum, silicon
Bangladesh Situation	Dominated by informal sector	No formal PV recycling yet



Solar PV module degradation and recycling process



E-waste degradation and recycling process

6. Specific challenges in Bangladesh:

Bangladesh encounters major difficulties in handling both E-waste and PV waste, stemming from a variety of issues. These issues include -

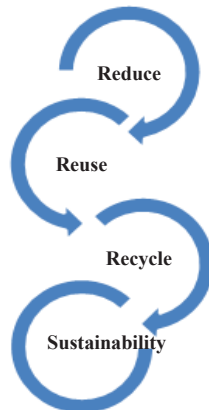
- Widespread informal e-waste recycling with child labor
- No national database on PV systems' lifespan or failures
- Public unawareness about toxic waste hazards
- Fragmented responsibility between ministries (Environment, Power, ICT)

7. Policy & Regulation (2000–2024):

In 2011, Bangladesh created regulations for E-Waste management, yet the enforcement is lacking. Established policies, guidelines, or databases for PV waste are totally absent. Solar panels importers and distributors are not responsible for collection or recycling. This absence of policy planning could transform Bangladesh's solar achievements into an environmental challenge by 2030.

8. Opportunities and Recommendations:

The "3R" framework for managing waste comprising Reduce, Reuse, and Recycle is essential for handling both E-waste and PV waste. When implemented in a prioritized sequence i.e. starting with reduction, followed by reuse, and finally recycling, these strategies assist in reducing environmental harm and fostering sustainability.

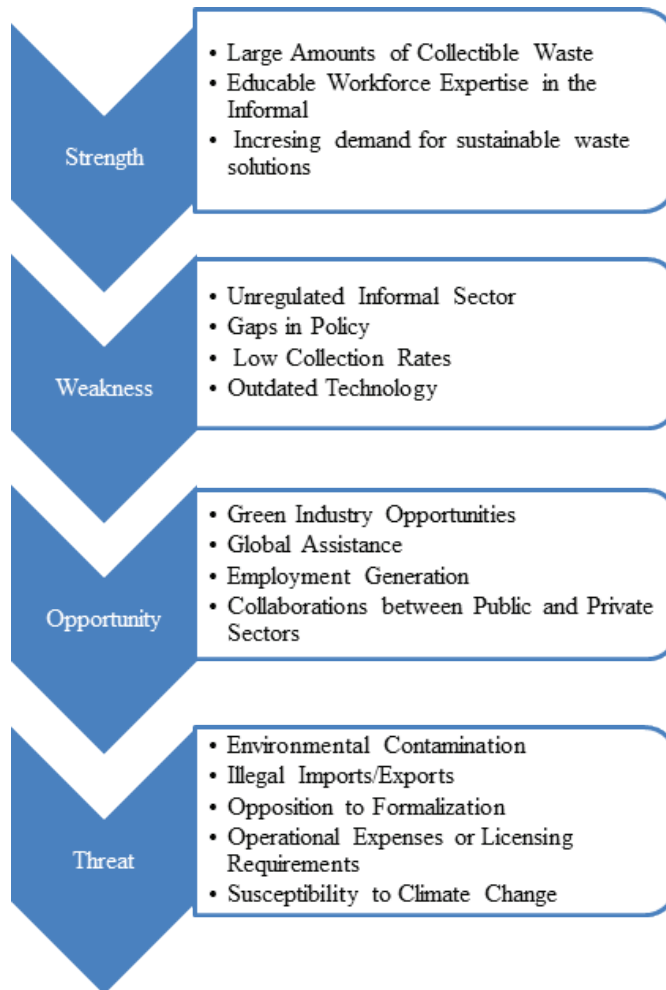


By successfully applying the 3Rs, we can shift from a linear "take-make-dispose" framework to a circular economy that reduces waste and enhances resource use.

By adopting the 3Rs and promoting a circular economy perspective, Bangladesh has the potential to turn its waste management issues into prospects for economic advancement, social progress, and environmental sustainability.

SWOT Analysis of PV Waste and E-Waste Management in Bangladesh:

Bangladesh has a significant opportunity to develop a robust and sustainable PV and E-waste management system. Addressing the identified weaknesses and capitalizing on the opportunities through strategic planning and action will be crucial for realizing the full potential of resource recovery, economic growth, and environmental protection.



To achieve sustainable development, it is crucial to adopt effective strategies and measures for the management of E-waste and pv waste. By executing the strategies and actions outlined below, we can progress toward a more sustainable future by managing E-waste and PV waste in a way that reduces their environmental impact and fosters a circular economy.

Strategy	Action
Legislation	Update and enforce E-waste rules; add PV under hazardous waste list
Awareness	National campaigns on E-waste and pv risks
Infrastructure	Set up pilot recycling centers
Solar sector reform	Mandate take-back or Extended Producer Responsibility (EPR) for panel suppliers
Research	Funding for studies on environmental impact of expired solar panels

9. Conclusion:

E-waste and photovoltaic PV waste represent two aspects of Bangladesh's growth journey. While E-waste is already posing dangers to health and the environment, PV waste is on the horizon as the first generation of solar systems reaches the end of its life. Bangladesh must learn from its previous errors and not allow history to repeat itself. An anticipatory approach that encompasses regulation, recycling, and public education has the potential to transform this challenge into an opportunity for achieving sustainable development and mitigating global warming as part of a green and equitable transition. Therefore, it is essential to establish a dependable and sustainable policy to optimize the management of E-waste and PV waste in Bangladesh.

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